Scratching the surface

The epithelial surface is the site at which we first encounter pathogens. Two recent advances indicate that intraepithelial lymphocytes use specialized tools to help protect this surface.

The skin and the digestive, respiratory and genitourinary tracts are covered by epithelia. Epithelia serve as selective borders that allow for the orderly transit of water, ions, gases, nutrients and waste products, but protect against the entry of pathogens. Epithelial cells thus provide a critical physical barrier, separating us from our surroundings. Many epithelia contain specialized T cells — intraepithelial lymphocytes — which in many cases derive from different precursors, and have different T-cell receptor repertoires, than other T cells. Despite these differences, intraepithelial lymphocytes behave similarly to other T cells in many *in vitro* assays of T-cell function. Emerging evidence, however, indicates that intraepithelial lymphocytes have unique properties that help them to interact with epithelial cells. In particular, recent results from several laboratories are beginning to provide a detailed understanding of two mechanisms that specific interactions between intraepithelial lymphocytes and epithelial cells.

The first important discovery came from a series of experiments designed to identify and characterize molecules that might be selectively expressed on the surface of intraepithelial lymphocytes. In the 1980s, several groups produced monoclonal antibodies that reacted with intestinal intraepithelial lymphocytes from various species. Several of these antibodies, including HML-1 and M290, are now known to recognize the integrin $\alpha^E\beta_7$ (also known as cell-surface marker CD103) [1,2]. $\alpha^E\beta_7$ is expressed on nearly all intestinal intraepithelial lymphocytes, and on many lymphocytes in the lamina propria, just below the epithelium (Fig. 1a); lymphocytes within or immediately adjacent to respiratory or reproductive epithelia also frequently express $\alpha^E\beta_7$.

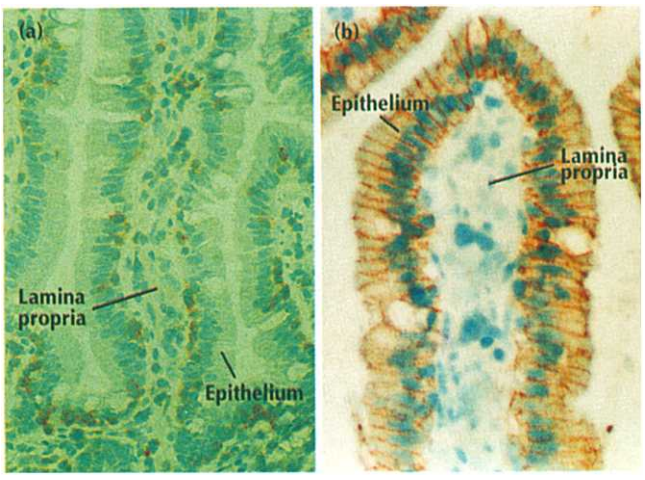

Fig. 1. Expression of integrin $\alpha^E\beta_7$ and E-cadherin in the human small intestine. Sections through villi of normal human small intestine were stained with antibody to **(a)** $\alpha^E\beta_7$ or **(b)** E-cadherin. $\alpha^E\beta_7$ is expressed on lymphocytes in the epithelium and lamina propria, but not on epithelial cells. E-cadherin is expressed on the basolateral surface of epithelial cells. (Photomicrographs courtesy of Michael Brenner and Gary Russell; reproduced with permission from [9] and [13].)

Mouse skin intraepithelial lymphocytes uniformly express $\alpha^E\beta_7$ at a high level [3]. These 'dendritic epidermal T cells' derive from a unique thymic precursor, have an unusual morphology and express a single, essentially invariant $\gamma\delta$ T-cell receptor. No such population is found in the human epidermis, and T cells in the human dermis do not normally express $\alpha^E\beta_7$. For the most part, T cells at non-epithelial sites are much less likely to express this integrin. For example, $\alpha^E\beta_7$ is expressed on only ~3–5% of mouse thymocytes and only ~1–3% of human blood T cells. Mast cells, which are abundant in the vicinity of some epithelia, express $\alpha^E\beta_7$ following activation *in vitro* [4], but B cells, granulocytes, and nonleukocytic cells are not known to be capable of expressing this integrin.

The identification of the HML-1/M290 antigen as a member of the integrin family provided important clues about possible functions of this molecule. Integrins are heterodimeric cell-surface glycoproteins that can mediate both cell–extracellular matrix and cell–cell adhesion. For example, the interaction of the lymphocyte integrins LFA-1 ($\alpha^L\beta_2$) and VLA-4 ($\alpha^4\beta_1$) with the endothelial cell ligands intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) is known to be crucial for the recruitment of these cells from the bloodstream into a variety of tissues. Ligand binding by integrins is known to involve both their α and β subunits.

The HML-1/M290 α subunit, α^E, is distinct from all other previously characterized integrin α subunits; it is not known to be capable of pairing with any integrin β subunit other than its HML-1/M290 partner, β_7. However, the β subunit, β_7, is able to associate with either of two integrin α subunits: α^E or α^4. This finding generated considerable interest, as $\alpha^4\beta_7$ has a special role in directing lymphocyte traffic; $\alpha^4\beta_7$ is a receptor for MAdCAM-1 (mucosal addressin cell adhesion molecule 1), a glycoprotein selectively expressed on endothelial cells in the gut and associated lymphoid organs [5]. This

adhesive interaction directs the selective recruitment (or 'homing') of a subset of blood T cells expressing high levels of $\alpha^4\beta_7$ to these sites.

It has long been hypothesized that intraepithelial lymphocyte precursors use a homing receptor to migrate selectively to the epithelium. The structural similarity of $\alpha^E\beta_7$ to other molecules involved in directing lymphocyte traffic fueled speculation that this integrin might be the intraepithelial lymphocyte homing receptor. To test this possibility, several investigators analyzed the effects of anti-$\alpha^E\beta_7$ antibodies on T-cell–endothelial cell adhesion *in vitro*, and on T-cell migration *in vivo*. The anti-$\alpha^E\beta_7$ antibodies do not seem to have effects in either system. While it is possible that these antibodies fail to block because they recognize epitopes of $\alpha^E\beta_7$ that are not involved in binding to the relevant ligand, these results did suggest that a search for other potential $\alpha^E\beta_7$ ligands was justified.

The first major breakthrough in the search for $\alpha^E\beta_7$ ligands came from the observation that the adhesion *in vitro* of intestinal intraepithelial lymphocytes to epithelial cells derived from intestine, breast, or lung could be inhibited by antibodies against $\alpha^E\beta_7$ [6–8]. Cepek and colleagues [9] have now made the exciting and unexpected discovery that $\alpha^E\beta_7$-mediated adhesion of human intraepithelial lymphocytes to epithelium *in vitro* is dependent upon the epithelial cell adhesion molecule E-cadherin (Fig. 1b). The evidence for this is as follows. First, antibodies against E-cadherin inhibit binding of intraepithelial lymphocytes to the same extent as antibodies against $\alpha^E\beta_7$, and the effects are not additive. Second, antibodies against E-cadherin have no effect on binding of lymphocytes that do not express $\alpha^E\beta_7$. Third, binding of intraepithelial lymphocytes to transfected cells expressing E-cadherin, but not to mock transfectants, is inhibited by antibodies against $\alpha^E\beta_7$. And fourth, binding of $\alpha^E\beta_7$-transfected, but not $\alpha^4\beta_7$-transfected, cells to epithelium is blocked by antibodies to E-cadherin.

Similar results have now been obtained using mouse intestinal intraepithelial lymphocytes and epithelial cells [10]. Although these data do not prove a direct interaction between $\alpha^E\beta_7$ and E-cadherin, they do strongly suggest that these molecules may be counter-receptors. This comes as a surprise, as 'classical' E-cadherin-mediated interactions are both homotypic and homophilic: an E-cadherin molecule on the surface of an epithelial cell acts by binding to another E-cadherin molecule on the surface of an adjacent epithelial cell (Fig. 2). (Adhesion of intraepithelial lymphocytes to epithelial cells cannot be mediated by homophilic E-cadherin binding, as intraepithelial lymphocytes do not express E-cadherin.) The $\alpha^E\beta_7$–E-cadherin interaction is the first known interaction between members of these two major families of cell adhesion molecules.

There are at least three possible explanations for the high frequency of $\alpha^E\beta_7$ expression on intraepithelial lymphocytes. First, it seems likely that the $\alpha^E\beta_7$–E-cadherin interaction acts to favor the retention of T cells expressing $\alpha^E\beta_7$ in the epithelium. This would provide a mechanism for accumulating lymphocytes in specific tissues that does not depend on 'homing' (a term best reserved for processes involving organ-specific lymphocyte–endothelial cell interactions). Second, it is possible that $\alpha^E\beta_7$-expressing intraepithelial lymphocyte precursors are selectively recruited to epithelia; this possibility is supported by the recent finding [3] that a subset of thymic intraepithelial lymphocyte precursors do express $\alpha^E\beta_7$. However, it is not yet known if thymocyte $\alpha^E\beta_7$ expression is restricted to intraepithelial lymphocyte precursors. (An alternative possibility is that $\alpha^E\beta_7$ is required at a particular stage of thymic differentiation of intraepithelial lymphocytes as well as other T cells.) The selective recruitment of $\alpha^E\beta_7$-expressing cells could be mediated by $\alpha^E\beta_7$ itself or by another, as yet unidentified homing receptor. The third possible explanation for the high frequency of $\alpha^E\beta_7$ expression on intraepithelial lymphocytes is induction *in situ*. Blood or lymph node T cells can be induced to express $\alpha^E\beta_7$ by exposure to the growth factor TGF-β, which is produced by epithelial cells [1]. In addition, it has now been shown that immunoglobulin-E-mediated activation of mast cells causes the release of soluble factor(s) that induce $\alpha^E\beta_7$ expression by T cells [4]. The ability of soluble factors to

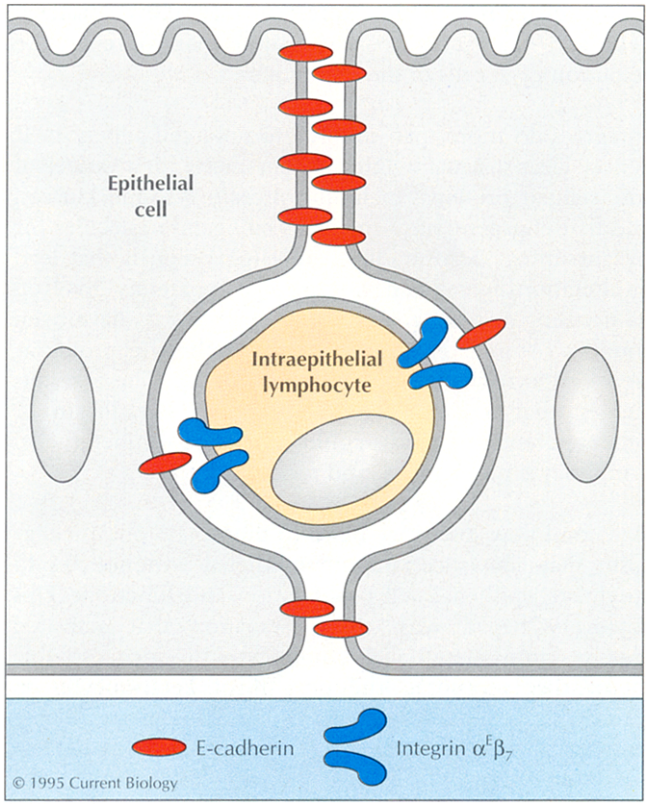

Fig. 2. Adhesive interactions mediated by integrin $\alpha^E\beta_7$ and E-cadherin. Epithelial cell adhesion to adjacent epithelial cells is mediated by the homophilic interaction of two E-cadherin molecules. Epithelial cell adhesion to intraepithelial lymphocytes is mediated by both E-cadherin and $\alpha^E\beta_7$. This is likely to involve heterophilic binding of $\alpha^E\beta_7$ to E-cadherin (see text).

induce changes in $\alpha^E\beta_7$ expression may allow epithelial cells and mast cells to exert some control over the retention of T cells in the epithelium.

Integrins do more than simply promote cell adhesion. It is now clear that many integrins interact with a variety of intracellular proteins, including protein tyrosine kinases, and that engagement of integrins by ligand molecules (or by anti-integrin antibodies) can trigger signals that lead to alterations in cell behavior. $\alpha^E\beta_7$ is apparently involved in signaling as well, as antibodies against $\alpha^E\beta_7$ have been found to stimulate intraepithelial lymphocyte proliferation and cytolytic activity *in vitro* [3,11]. These results suggest that $\alpha^E\beta_7$-mediated binding to E-cadherin or other potential ligands could influence intraepithelial lymphocyte proliferation and function *in vivo*.

A second new discovery helps to illuminate one mechanism that allows certain intraepithelial lymphocytes to modulate epithelial cell proliferation and function. This discovery began with the observation that activated mouse skin intraepithelial lymphocytes produce a soluble factor that promoted the growth of keratinocytes in culture [12]. The factor was identified as keratinocyte growth factor (KGF), known to be required for normal growth and repair of epidermis. KGF also promotes the proliferation of epithelial cells other than keratinocytes, and further investigation revealed that it is also produced by intraepithelial lymphocytes from mouse intestine. Strikingly, KGF was found to be produced only by intraepithelial lymphocytes expressing the γδ T-cell receptor: intraepithelial lymphocytes expressing the αβ T-cell receptor, and non-epithelial T cells expressing both γδ and αβ T-cell receptors, could not produce KGF. After skin wounding, other cells in the dermis are likely to produce more KGF than do intraepithelial lymphocytes, but KGF produced by intraepithelial lymphocytes may be especially important as it is released directly within the epidermis. In addition, intraepithelial lymphocytes might produce KGF in response to some stimuli that fail to induce KGF production by other cell types.

The discoveries of integrin $\alpha^E\beta_7$ and of KGF-producing intraepithelial lymphocytes are important early steps toward an understanding of the functional differences between intraepithelial lymphocytes and other T cells. These discoveries indicate that intraepithelial lymphocytes have specialized mechanisms for communicating with epithelial cells. It will be important to determine how these mechanisms contribute to a coordinated response to infection and wounding of the epithelium. Valuable insights are likely to come from mouse experiments, such as studies of α^E gene knockout mice, that directly address the importance of specific mechanisms *in vivo*. As there are important cross-species differences in intraepithelial lymphocyte ontogeny and phenotype, additional studies will be required to determine how these insights apply to other species, including humans.

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